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01 APR 1997
LONDON

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation — 1735870002

4. Title of the invention

Invention

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

Kilburn & Strode
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Form 51/77 26.2.98
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INVENTION

The present invention relates, *inter alia*, to the amplification of nucleic acid samples.

1. Summary

The invention provides a method to obtain distinct immobilized areas (hereafter called "colonies") containing a large number of identical copies of an initial, unique, nucleic acid fragment (i.e., a DNA or an RNA fragment, hereafter called "template"). Various embodiments of the invention are set out in the following description and also in the accompanying claims.

The method of the invention allows to obtain colonies ranging from a few nanometers to several micrometers in size with a spacing between colonies ranging from zero to several millimeters. Depending on the embodiment of the method, these colonies can be periodically, specifically or randomly arrayed and have two or three dimensional shapes, with the possibility of having any possible combination of these properties. The method consists in using an homogeneous mix of one, two or more different oligonucleotides which are immobilized with an appropriate density by linkage to an appropriate matrix. The templates of interest have to contain the (5'-3') sequence of one of the primer followed, after up to several thousands of unspecified nucleotides, by the complementary and inverted sequence of the second primer. The colonies are obtained by performing a PCR reaction, where the immobilized oligonucleotides take the place of the primers. The density of colonies can be controlled by the initial concentration of the template fragments and the timed allowed for annealing before an optional washing step is performed. The size of the colonies can be controlled by the total number of PCR cycles, i.e., those before and after the optional washing step. Depending on the embodiment of the method, these arrays can be used to analyze the sequence of nucleic-acid fragments (e.g., sequencing), to compare the

sequence of different nucleic-acid fragments (e.g., polymorphism detection and screening), to sort out and compare the amounts of numerous different nucleic-acid fragments mixed in a solution (e.g., gene expression monitoring) with unprecedented throughput and without using electrophoretic separations ("gels") or previously described methods based on oligonucleotide chips (however, in some embodiments of this method, we describe how to use an oligonucleotide chip for colony generation).

2 Field of the invention

This invention addresses generally the field of arraying nucleic acids (or molecules tagged with nucleic acids) samples onto a solid phase matrix for use in the field of sorting, amplifying, comparing and identifying nucleic acids or molecules and objects tagged with nucleic acids.

3 Background

3.1 Introduction

Molecular biology and pharmaceutical drug development make now intensive use of nucleic acid analyses. The most challenging areas are nowadays whole genome sequencing, polymorphism screening and gene expression monitoring. Currently, up to hundreds of thousands of samples are handled in single projects. This capacity is limited by the available technology. Project like the "human genome project" (DNA sequencing) and finding genes involved in rare diseases imply to handle millions of samples. In most cases, it is simply not possible to decrease indefinitely the time to process a single sample. An

alternative is then to perform many processes in parallel. The introduction of robots, 96 and 384 well plates, high density gridding machines and recently high density oligonucleotide arrays are starting to bring answers to the demand in ever higher throughput. Such technologies allow to handle simultaneously 50,000-100,000 samples simultaneously within days and even hours.

In most of the existing and envisaged methods to perform nucleic acid analyses, it is necessary to first extract the nucleic acids of interest (e.g., genomic or mitochondrial DNA or messenger RNA (mRNA)) from the organism. Then it is necessary to isolate the nucleic acids of interest from the mixture of all nucleic acids and usually, to amplify these nucleic acids to obtain quantities suitable for their characterization and/or detection. Note that isolating the nucleic fragments is necessary even when one is interested in a representative but random set of all of the different nucleic acids, for instance, a representative set of all the mRNAs present in a cell or of all the fragments obtained after the genomic DNA has been cut randomly into small pieces. The characterization of the isolated nucleic acids can be made by many different methods depending on the goals of the project. There are now methods in which it is possible to use the same assay (reactants and detection system) on all the samples simultaneously. Such methods are well suited to take advantage of samples which have been arrayed on a solid surface. The present invention will be relevant for such methods. Therefore, we will review state of the art for arraying amplified DNA samples onto solid surfaces.

3.2 Biological amplification

Several methods can be used to amplify DNA with biological means and are well known by the men of art. Basically, the fragments of DNA have

first to be linked to a "vector" with the use of nucleic acid cutting and binding enzymes. This "cloned DNA fragment" is then introduced into the biological host by means of well established protocols. By growing the host under the right conditions, the host multiplies and simultaneously multiplies the cloned DNA fragment. Usually, the hosts are randomly spread over a growing media (agar plates) in a density leading to colonies which started from individual host. Up to millions of simultaneous amplification of isolated DNA fragment can be carried out simultaneously. The density of colonies is of the order of 1 colony/mm^2. To use this system, one option is to transfer the biological host colonies to a membrane, and to recover the DNA from within the biological host. Transferring to the membrane can be done by replication, where the density of colonies is conserved, or by means of gridding robots (see below). With this option however, the amount of transferred DNA is limited and often insufficient for non radioactive detection. Another option is to transfer individually each colony into a container (e.g., 96 well plates) where the growing can be pursued until enough amount of DNA has been produced. The amplified DNA fragment has to be recovered from the biological host with an appropriate purification process, which is generally time and labor consuming, and presently difficult to automate.

3.3 Polymerase Chain Amplification

This revolutionary technique has been proposed by Mullis and is now well known by the men of art. In this amplification process, the DNA fragment of interest, hereafter called "template", can be amplified using two short (typically 20 base long) oligonucleotides which flank the region to be amplified, and which are usually called "primers". Amplification occurs during the PCR cycling, in fact repeating a denaturing step (the reaction mix is heated to, e.g., 95°C in order to

separated double stranded fragments into two single stranded fragments), an annealing step (the reaction mix is brought to e.g., 45° c to allow the primers to anneal to the single stranded templates) and an elongation step (copies of the templates are realized by nucleotide incorporation at the ends of the primers with the DNA polymerase enzyme). This amplification can be done in solution, i.e., neither the primers nor the template are linked to any solid matrix. More recently, it has been proposed to use one primer grafted to a surface in conjunction with free primers in order to simultaneously amplify and graft the PCR product onto the surface. In these methods, the amplification is performed in containers (e.g., in 96 well format plates) in such a way that each container contains the PCR product(s) of one reaction. In this approach, some of the PCR product end up homogeneously grafted on the whole surface of the container which has been in contact with the reactant during the PCR cycling. The grafting simplifies subsequent assays and allows thorough automation.

3.4 Sample arraying

When the PCR products are linked to the container where the amplification took place, one can consider this as a direct arraying process. The density of the array is then limited by the available vessel, currently only in 96 well plate format, or ~0.02 samples/mm^2.

Arraying of DNA samples is more classically performed on membranes (e.g., nylon or nitro-cellulose). With the use of suitable robotics (e.g., Q-bot) it is possible to reach a density of up to 10 samples/mm^2. In this case, the DNA is covalently linked to the membrane by physicochemical means (e.g., UV irradiation). These technologies allow to array both DNA templates (typically more than 100 base pair

long fragments) and DNA probes (oligonucleotides). Gridding up to 100,000 samples on a single membrane have been reported.

New approaches based on pre-arrayed glass slides (arrays of reactive areas obtaind by ink-jet technology or arrays of reactive polyacrylamide gels) allow to array up to 100 samples/mm^2. With these technologies, only probe (oligonucleotide) grafting has been reported. Reported number of samples are still fairly low (64 to a little higher) but the techniques are certainly suitable for large scale up (-1,000,000 samples).

Higher sample densities are achievable by the use of "biochips" obtained with the use of micro-lithographic techniques Currently, chips with 625 probes/mm^2 are used in applications for molecular biology.

Currently, up to 65536 different oligonucleotides can be arrayed on a single chips. Presently, these chips are manufactured by direct solid phase oligonucleotide synthesis, and thus this technology is restricted to the production of probe chip.

To summarize, in the present state of the art, there is no methods which allows for simultaneous amplification and arraying of template DNA. Amplification has to be carried out in a biological host or by PCR, and arraying has to be performed in an additional step.

Various preferred embodiments of the present invention will now be described in greater detail.

4 Detailed description of the invention

4.1 Definitions :

This invention description uses the jargon of the men of the art of molecular biology, unless a more restrictive or a more general definition of a term has been explicitly given. In particular, the PCR

jargon (annealing, denaturing, elongation, etc..) will be used without further explanation.

Nucleic acid : DNA or RNA molecules of any size and composition, single stranded or double stranded, in any derived chemical form (including, without being exhaustive, isotopic analogs, fluorescently labeled analogs, chemically reactive analogs, biotinylated analogs, chemically labeled analogs) and any analog capable of specific or non specific binding to nucleic acids and which allow or do not allow initiation of nucleic acid duplication with the use of DNA or RNA polymerase enzyme.

Oligonucleotide : a nucleic acid of typically, but not restricted to, 5 to 50 bases in size, single stranded or double stranded, obtained by biological, biochemical or chemical means.

Assay : an assay designed to identify, characterize and dose a nucleic acid.

Template : refers to the nucleic acid which is to be assayed.

Probe : refers to the nucleic acids which are used to assay the template.

Primer : refers to nucleic acid fragments which allows the initiation of template and/or probe replication with the use of a DNA or an RNA polymerase.

Support : refers to the material to which it is possible to graft by any mean (including, without being exhaustive, chemical, biochemical, physical mean). The support might be the surface of a solid material, a bulk porous material, a polymeric solution or a colloidal solution of any size or shape. The support can be chemically, biochemically or physically functionalized (typically with free -NH_2 capable of reacting with phosphate on oligo). This includes functionalization with one or several different nucleic acids, where the different nucleic acids can be homogeneously mixed and/or grouped by their

Colonies : distinct immobilized areas of the support containing a large number of identical copies of an initial, unique, nucleic acid fragments.

4.2 Preparation of the support

This invention involves to graft homogeneously one, two or more different primers to the support or to parts of the support. In this grafting process, it is essential that the 3' end of the primers are left accessible for priming strand extension. Any method meeting this requirement is potentially suitable for preparing the support and the invention is not restricted to the few examples given below. Such a method has been described by Nunc on commercially available vessel. Grafting to silanazed glass has been described. Grafting biotinylated primers to supports covered with streptavidin is an alternative. In these methods, one, two or more different primers can be grafted homogeneously and simultaneously. Direct synthesis of oligonucleotides onto a solid support with the 3' end free is under development. In such a method, micro-lithographic methods can be used to simultaneously synthesize many different oligonucleotides, but in distinct areas of some micro-meters in size. Within each area, only one type of non degenerated oligonucleotide can be homogeneously synthesized. An homogeneous mix of degenerated oligonucleotides (i.e., a mix of oligonucleotides differing only in one or a in few positions in their sequence) can however be synthesized in each area, leading to the case of two or more different grafted primers. Each area carrying only one type of nucleotide can however be specifically modified to carry two or more totally different primers. One way to achieve this is to use secondary primers with the 3' half of their sequence complementary to the grafted primer, and the 5' half with a different sequence, each

carried out by allowing the secondary primers to specifically hybridize with the grafted primers, and by extending the grafted primers with a DNA polymerase. This will result in an homogeneous mix of different primers on each area, with the ability to specifically modify each different area with different sets of secondary primers.

4.3 Preparation of the template

The template has to be flanked with 2 oligonucleotides, defined in reference to the sequence of the set of grafted oligonucleotides : the sequence of one of the grafted primers can be present on the 5' end of the template and the reverse complement sequence of one of the grafted primers can be present at the 3' end of the template. The template preparation can be done either by biological means (cloning the template in the appropriate vector) or biochemical means (ligating the template to the appropriate oligonucleotides).

4.4 Primary growing of the colonies

4.4.1 Initializing the colony growing

Growing the colonies is performed through PCR cycling the support. The PCR conditions to be used here are the same as in classical PCR where the grafted primers take the place of the primers normally found in solution. One can distinguish between a colony initiating step and a colony growing step. In the initiating step, the template molecules which are in solution anneal with the primers grafted on the support at annealing temperature (e.g., 50° C). The annealed templates are duplicated by primer elongation using classical PCR conditions. After sufficient time (e.g., 30 seconds), the support is heated to a

the template is washed away and replaced with fresh PCR reaction mix without primers and without template. The density of template molecules which anneal to grafted primers will depend on the concentration of template molecules and on the duration of the annealing. Changing the PCR reaction mix is however optional. To increase the number of initiated colonies, one might choose to keep template present in the PCR reaction mix during part of or during the whole colony growing step. Also, to reduce non-specific priming problems and control the colony initiating time or synchronize the colony initiation between samples, one might choose to start the colony initiation by a denaturing step.

4.4.2 Growing the colonies

Colony growing is performed by PCR cycling the support after the initiating cycle(s). The number of duplicated templates present in the colony will increase with the number of PCR cycles. The size of the colony will depend on the number of duplicated templates and presumably on many other parameters, including, but not restricted to, the micro-structure and the geometry of the support, the density of grafted primers, the length and the stiffness of the template molecules and certainly on many other physical and chemical parameters (temperature, ionic strength, viscosity, etc...).

4.5 Secondary growing of colonies

Here we describe how to prepare existing colonies for using them as a new support for generating PCR colonies from new template samples. First, after colony growing, a single stranded specific exonuclease might be used to remove all primers which had not been elongated, or in other words, which are not part of a colony. Secondly and independently, the DNA molecules forming the colonies can be cleaved with the use of endonucleases (e.g., restriction enzymes) and then

denatured and washed. Alternatively, the double stranded DNA forming the colonies can first be denatured and then be partly digested with single stranded specific exonucleases or with exonuclease which digest only one strand in a double stranded. In all cases, these treatments result in single stranded fragments grafted onto the support and which can be used for new PCR colony growing if the adequate template is used for initiation. Note that not only prepared template can be used for colony initiation, but also unprepared nucleic acid fragments which present sequence homologies with the templates which formed the primary colonies. Note also that now the maximum size of the secondary colony is restricted to the size of the primary colony onto which it grows. Note that the process of secondary colony growing is recurrent and several secondary growing processes can be used sequentially to create colonies for specific applications.

4.5 Random colony replication

Here we describe how to randomly replicate primary or secondary colonies. First, it is necessary to use the support with existing colonies and to perform PCR cycling with the suitable primers in solution but no template in solution. In this way, one will duplicate the templates forming the colonies and recover the duplicated templates in solution. One option is to use the same primers as those originally grafted on the support. In that case, one obtains a collection of copies of all the templates present on the support. An alternative option is not to use all the primers originally grafted on the support but to mix some of them with specific primers or simply to use only specific primers. In that case, it is possible to specifically duplicate only a partition of the templates which formed colonies on the support. Note the duplication can be done several times. The duplicated

this way, this replication process allows to create indefinitely new sets of colonies which have statistically the same distribution of templates as the original set of colonies. This process can clearly be used recurrently with the primary and secondary growing of colonies

4.6 Experimental evidence

The invention has been successfully tested. A mix of two oligonucleotides, which are phosphorilated at the 5' end, have been grafted on 96 well Nucleolink plates from Nunc. These plates are specially formulated for allowing the covalent grafting of 5' phosphorilated DNA fragments through a standard procedure. A template has been cloned in a vector with the appropriate DNA sequence at the cloning site, and 174 bp long linear double stranded DNA template has been obtained by PCR amplification. The template PCR product has been purified on Qiagen columns in order to remove the nucleotides and the primers used during the PCR amplification. The purified template (in solution in PCR buffer with A,T,G and C nucleotides and Taq Gold polymerase) has been spread on the support and 50 PCR cycles have been performed. Several different concentration has been tested and for each sample a control reaction realized without DNA Taq polymerase has been performed. Each sample has been stained with YO-PRO (Molecular Probes), a standard highly sensitive stain for double stranded DNA. (Visualisation can be done e.g. with (1) biotinylated nucleotides - visualise with streptravidin - fluorescent bead or (2) intercalating dye (e.g. ethidium bromide)). The resulting products have been observed on a confocal microscope with appropriate excitation and detection filters (the bottom of each well is flat and allows observation with an inverted fluorescence microscope). Typical observations are: in a control well (without polymerase), only rare objects can be observed on a blank surface. These objects have an irregular shape, are 20 to 200 micro-meters in size and have a thickness much larger than the field depth of the observation. In

a well where DNA polymerase was present, in addition to the objects of irregular shape observed in the control well, a great number of fluorescent spots can be observed. They present a circular shape, they are 1 to 5 micro meters in size and do not span over the depth of view of the observation. The number of spots depends clearly on the concentration of the template. From the observed size of the colonies, it was estimated that more than 10,000 distinct colonies can already been arrayed within 1 mm^2 of support.

5 Applications

The invention described in section 4 can be used for different applications. Here we present a few possible embodiments of the invention in order to illustrate its usefulness and novelty. The invention is clearly not restricted to these examples. However, these embodiments are also covered. The definitions given in section 4 apply in the following descriptions.

Preparation of arrays of genomic DNA

The first step is to extract the DNA from the biological host and cut it randomly into "small" (50 to 10,000 bases long) pieces by any mean known by the men of art (e.g., a phenol-chloroform extraction followed by ultrasound treatment). In order to standardize the experimental conditions, the extracted and cut DNA fragments can be sorted out against their size, e.g., by agarose gel electrophoresis, in order to reduce the variability in size of the template. Secondly, the extracted, cut and (optionally) sorted template DNA fragments are tailed with DNA fragment containing the sequence of the primers which are grafted on the support. Alternatively, the template DNA fragments can be cloned the into a biological vector which

which are grafted on the support. This cloned DNA can be amplified within the biological host and extracted. Hereafter, we will designate the DNA fragments obtained after such a suitable process by the expression : "prepared genomic DNA".

5.1 Random arraying for highly parallel base by base shot-gun sequencing

A sequencing method has been recently described for massively parallel base-by-base sequencing (Brenner,s Fok 1 approach). This method is designed to work on millions of randomly arrayed samples of templates. Here we present an embodiment of our invention for randomly arraying up to billions samples of DNA in a tractable fashion. The fragments used to tail the template have to be suited for the sequencing method to be used. For instance, if the method described in Brenner's Fok 1 invention, a correctly oriented and positioned Fok I recognition site has to be included. Practically, the tailing of the template can be achieved by ligation of the templates within a biological vector and subsequent biological amplification and DNA extraction, or by ligation of two oligonucleotides corresponding to the primers and subsequent PCR amplification and purification. After amplification and purification, the bulk mix of templates is spread on the support (with PCR buffer, nucleotides and DNA polymerase). The support covered with the template/PCR mix solution is submitted to PCR cycling. The exact embodiment of the cycling will depend on the shape and nature of the support. For instance, if Nucleolink 96 well format support is used, a standard PCR cycler can be used. If the support is made of "small" plates (1-2 cm), *in situ* PCR cyclers can be used. Larger plates can be used. The cycling is then performed by dipping the setup in water baths held at the different temperatures required for PCR cycling. Considering the sample density already observed in section 4.6, i.e., 10,000 colonies/mm^2, up to approximately 500,000 samples can be spread on the bottom of 1 Nucleolink well, approximately 50,000,000 samples can be spread on a full 96 well Nucleolink plate, and 1,000,000,000 samples can be spread on an approximately 30x30 cm dedicated plate or on 20 96 well plates. Assuming that the setup used is designed to cover the plates with a 0.5 mm thick solution layer, which is not technological challenge, 25 micro-liter, 2.5 ml and 50 ml of PCR mix solution can be used, respectively. Note that in that process only one bulk sample

need be processed throughout the whole process. Thus no high throughput robotics are required. Only for using dedicated plates, a dedicated PCR cycler might be used although existing equipment is already suited, with little effect on the overall throughput. Assuming the base by base sequencing method described in Brenner/Lynx patent is used, typically 20 bases are to be sequenced on each sample. Thus, using one 96 well plate, 1,000,000,000 bases of raw sequence could be sequenced with one template preparation. Using a 10x coverage shot gun approach to sequence the entire human genome, the arrays of sample required to obtain the raw sequence data could be obtained using as little as thirty 96 well plates and 75 ml of PCR reaction mix. This has to be compared to the amount of reactants (of comparable cost that those used in a PCR reaction) that are required to obtain the Sanger sequencing reaction products for use in the most widely used classical approach (ABI equipment based sequencing) : a 10x coverage shot gun approach on the human genome would require of the order of 60,000,000 sequencing reactions of currently at least 10 micro-liters each, thus 600 liters of reactants, or 8,000x more than what is required with the present invention. Here, the reactants required for the base-by-base sequencing method are not considered. However, it can be estimated that the amount of reactants in such a method are small, possibly comparable to the amounts required in our invention.

5.2 Random arraying of many samples for highly parallel base by base sequencing or fingerprinting by oligonucleotide hybridization

5.3 Directed arraying on DNA chips for highly parallel base by base sequencing of many samples

5.3 Random arraying for detection and quantification of mRNA from many samples

Gene expression monitoring is a tool of growing importance in molecular biology and in the field of understanding the molecular basis of diseases. Many methods have been proposed for direct or differential monitoring of gene expression. These methods are all more or less limited in the dynamical range of levels of expression which can be monitored, in the number of genes which can be simultaneously monitored and in the complexity and throughput of the methods. The use of PCR

colonies based techniques overcomes these problems and opens new opportunities which are unique to our invention. The possibility of making secondary colonies can be used.

One first step is to make PCR colonies from genomic DNA or from cloned genes. For example, starting from genomic DNA, the method proposes to cut randomly the genomic DNA into small Pieces (e.g. by treating the DNA solution with ultra-sounds), sort the molecules against their size and recover DNA fragments within a given range (e.g., around 1,000 bp), tail the recovered fragments with oligonucleotides corresponding to the primers grafted on the support for PCR colonies.

5.3 Directed arraying on DNA chip for detection and quantification of mRNA from many samples

5.4 Random arraying for standardized characterization of unknown expressed genes

Various aspects of the present invention are illustrated (without limitation thereof) in the accompanying figures, wherein:

Figure 1 illustrates the overall principle of the invention, with repeating steps of annealing, elongating (primer extension) and denaturing (separating of annealed strands).

Figure 2 illustrates one use of the present invention in whole genome anonymous gene expression monitoring. Primer PCRC p1 is shown as a white box and a different primer (PCRC p2) is shown as a black box.

Claims

1.A method of nucleic acid amplification, comprising the steps of :

A. providing a plurality of primers that are immobilised but that have one end exposed to allow primer extension ;
B. allowing a single stranded target nucleic acid molecule to anneal to one of said plurality of primers over part of the length of said single stranded nucleic acid molecule and then extending that primer using the annealed single stranded nucleic acid molecule as a template, so as to provide an extended immobilised nucleic acid strand ;
C. separating the target nucleic molecule from the extended immobilised nucleic acid strand;
D. allowing the extended immobilised nucleic acid strand to anneal to one of said plurality of primers referred to in step A) and then extending that primer using the extended immobilised nucleic acid strand as a template, so as to provide another extended immobilised nucleic acid strand;
E. separating the annealed extended immobilised nucleic acid strands from one another.

2. A method according to claim 1, further comprising :

F. using at least one extended immobilised nucleic acid strand to repeat steps D) and E), so as to provide additional extended immobilised nucleic acid strands and, optionally,
G. repeating step F) one or more times

3 .A method according to claim 1 or claim 2, wherein the plurality of primers is a plurality of primers that have the same sequence.

4.A method according to claim 1 or claim 2, wherein the plurality of primers comprises at least two different types of primer, one type having a different sequence from another type.

5.A method according to claim 4, wherein the plurality of primers consists of 2^n different types of primer, wherein n is an integer.

6.A method according to claim 4 or claim 5, wherein the different types of primer are present in substantially the same concentrations as one another

7. A method according to any preceding claim, wherein the primers are substantially homogenously dispersed over a given area

8. A method according to any preceding claim, wherein the primers are located in a predetermined arrangement (e.g. in a grid pattern)

9. A method according to any preceding claim, wherein a supply of nucleotides and a nucleic acid polymerase are used to extend primers

10 A method according to any preceding claim, wherein heating is used to separate annealed nucleic acid strands

11.A method according to claim 10 when dependent upon claim 9, wherein the nucleic acid polymerase is not rendered inactive by the heating conditions used to separate annealed nucleic acid strands.

12.A method according to claim 11, wherein said nucleic acid polymerase is *taq* polymerase , is another polymerase that is derivable from a thermophilic organism;or is a thermostable derivative thereof.

13.A method according to any preceding claim, wherein said primer extension results in the incorporation of one or more detectable labels (e.g. fluorescent labels or radiolabels) into extended immobilised nucleic acid strands.

14.A method according to any preceding claim, further including the step of treating one or more extended immobilised nucleic acid strands so as to release a nucleic acid molecule or a part thereof.

15. A method according to claim 14, wherein said treating consists of cleavage with a restriction endonuclease or with a ribozyme.

16. A method according to any preceding claim, wherein one or more of said primers has a restriction endonuclease recognition site or has part of such a site, which part becomes complete when primer extension occurs.

17. A method according to any preceding claim that is automated to allow repeated cycles of nucleic acid amplification.

18. A plurality of immobilised nucleic acids producable by a method according to any preceding claim.

19. The use of a method according to any of claims 1 to 17 or of a plurality of immobilised nucleic acid molecules according to claim 18 in providing amplified nucleic acid molecules for sequencing.

20. The use of a method according to any of claims 1 to 17 or of a plurality of immobilised nucleic acid molecules according to claim 18 in providing amplified nucleic acid molecules for diagnosis.

21. The use of a method according to any of claims 1 to 17 or of a plurality of immobilised nucleic acid molecules according to claim 18 in providing amplified nucleic acid molecules for screening

22 An automated apparatus for performing a method as described in any of claims 1 to 17; comprising a plurality of immobilised primers, a nucleic acid polymerase , a plurality of nucleotides and means for separating annealed nucleic acid strands.

23 An apparatus according to claim 22, wherein the means for separating annealed nucleic acid strands comprises a controlled heating means.

24. A kit for use in screening, diagnosis or in nucleic acid sequencing; comprising a plurality of immobilised nucleic acid according to claim 18

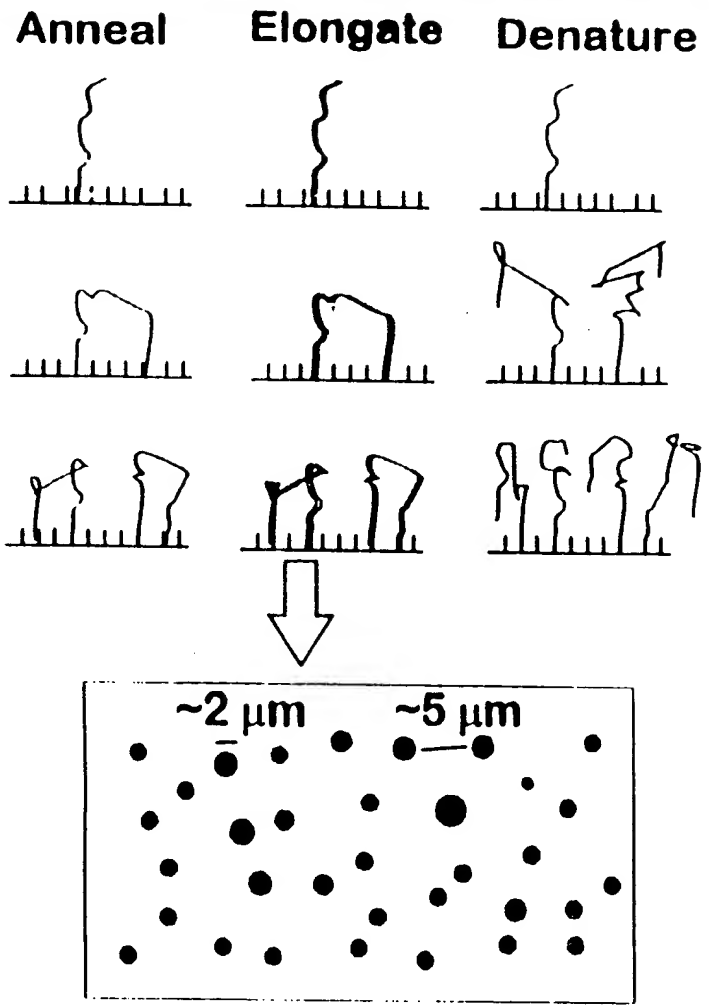
25. The invention substantially as hereinbefore described.

Abstract

A nucleic acid molecule can be annealed to an appropriate immobilised primer. The primer can then be extended and the molecule and the primer can be separated from one another. The extended primer can then be annealed to another immobilised primer and the other primer can be extended. Both extended primers can then be separated from one another and can be used to provide further extended primers.

FIGURE 1

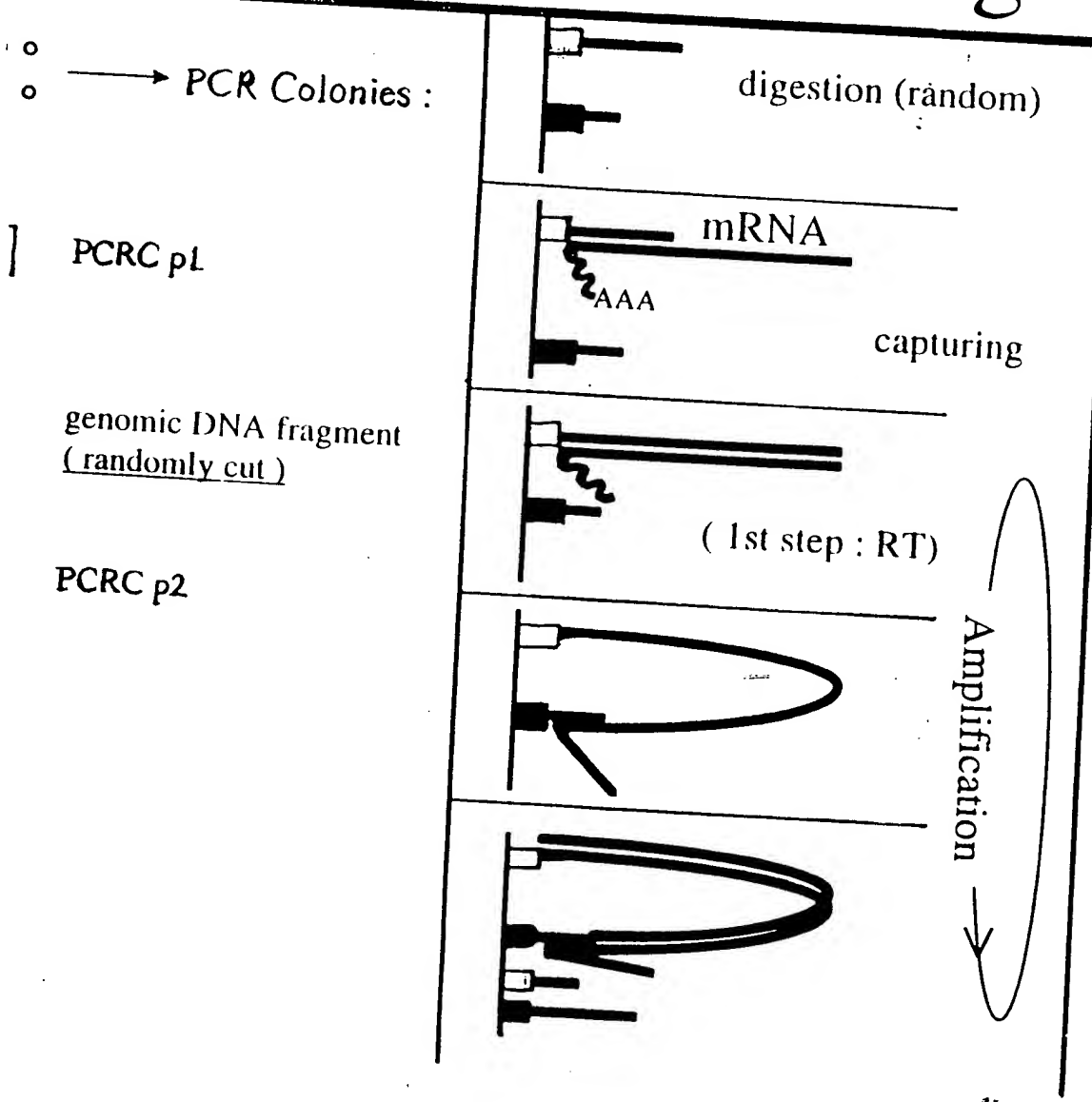
PCR colonies : principle



- typically, 4'000'000 colonies in 1x1 cm
- each colony is made of copies of the ***SAME*** initial DNA fragment

FIGURE2

Whole genome anonymous gene expression monitoring



- identification by fingerprinting + base by base sequencing
- No need to clone the genes
- No need to postulate ORFs

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